AMRL-TR-68-178

TOXIC EFFECTS IN MONKEYS EXPOSED TO 100% OXYGEN AT AMBIENT PRESSURE

MARILYN E. GEORGE
JAMES P. F. MURPHY
KENNETH C. BACK, PhD
Aerospace Medical Research Laboratory

CLARE C. JOHNSTON, PhD
IIT Research Institute

JULY 1969

AEROSPACE MEDICAL RESEARCH LABORATORY
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY *(Corporate author)* Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio* and IIT Research Institute, Chicago, Illinois**	2a. REPORT SECURITY CLASSIFICATION Unclassified 2b. GROUP N/A

3. REPORT TITLE

TOXIC EFFECTS IN MONKEYS EXPOSED TO 100% OXYGEN AT AMBIENT PRESSURE

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*

Final Report, January-April 1968

5. AUTHOR(S) *(First name, middle initial, last name)*

*Marilyn E. George
*James P. F. Murphy
*Kenneth C. Back, PhD
**Clare C. Johnston, PhD

6. REPORT DATE July 1969	7a. TOTAL NO. OF PAGES 28	7b. NO. OF REFS 16
8a. CONTRACT OR GRANT NO. F33615-68-C-1270 (In Part) b. PROJECT NO. 7163 c. 00 d. 005	9a. ORIGINATOR'S REPORT NUMBER(S) AMRL-TR-68-178 9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*	

10. DISTRIBUTION STATEMENT

This document has been approved for public release and sale; its distribution is unlimited.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY Aerospace Medical Research Laboratory, Aerospace Medical Div., Air Force Systems Command, Wright-Patterson AFB, OH 45433

13. ABSTRACT

Thirty-two monkeys were exposed to 100% oxygen at 750 mm Hg pressure for 4, 7, or 12 days, and the effects of this atmosphere on liver, kidney and lung morphology, kidney function, liver and kidney cellular respiration and energy production, and blood gas levels were studied. The mortality rate was approximately 40%. There was a mild uncoupling of oxidative phosphorylation and a decrease in ATP levels in liver and kidney tissue at all exposure times and a decrease in kidney function at 4 days with a return to normal levels by 12 days. However, the major effect was seen in the lung where the degree and time sequence of lung damage correlated fairly well with the blood Po2 levels. There were mild morphological changes at 4 days, with more severe effects at 7 days. At 12 days, four out of five monkeys had major lung damage whereas the remaining animal seemed to have only minor changes. The cause of death appeared to be hypoxia from severe diffuse lung damage.

DD FORM 1 NOV 65 1473

Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Oxygen Toxicity						
100% Oxygen						
Cellular Metabolism						
Blood Gases						
Kidney Function						

FOREWORD

This study was performed in support of Project 7163, "Research on Biomechanisms and Metabolism." The work was performed from January 1968 to April 1968 in the Toxicology Branch, Toxic Hazards Division. One phase of the work was done by personnel of the Illinois Institute of Technology, Chicago, Illinois, under Air Force contract F33615-68-C-1270.

The assistance rendered by Major V. L. Carter, Captain Richard Bradbury, Captain Roger Sopher, Captain Ethard Van Stee, Master Sergeant Joseph Young, Technical Sergeant Russell Colvin, Sergeant Doyle Manion, and Staff Sergeant Lewis Powers, is gratefully acknowledged.

This technical report has been reviewed and is approved.

C. H. KRATOCHVIL, Colonel, USAF, MC
Commander
Aerospace Medical Research Laboratory

SECTION I

INTRODUCTION

The use of a pure oxygen atmosphere for space flight has stimulated an intensive investigation into possible toxic effects of this environment. The severe pulmonary effects observed in animals breathing 100% oxygen at ambient pressure over a relatively short period of time have been well documented (Robinson, 1967; Kistler, 1965; Weir, 1961). Morphological studies indicate there is alveolar edema, exudate, atelectasis and fibrosis of the alveolar wall resulting in a thickening of the alveolar-capillary tissue barrier. Effects of this atmosphere on the morphology and metabolism of other organs also have been reported although the basic cellular and biochemical mechanism of oxygen toxicity has not been precisely defined.

Riesen et al (1966) have found an uncoupling of oxidative phosphorylation in mitochondrial preparations from the livers of rats after exposure to 100% oxygen at 760 mm Hg for 72 hours. These findings correlate well with morphological changes in liver cell mitochondria from the same group of rats reported by Schaffner (1965). Mautner (1966) described similar changes in mitochondria of kidney tissue from rats exposed for 1 and 2 days.

Since the projected atmosphere for some space flights is 100% oxygen at 258 mm Hg pressure, emphasis has also been placed on investigations into the possible toxicity of this environment.

Studies using various experimental animals have indicated that there are morphological changes, varying in intensity, in liver, kidney and lung tissue after exposure for 8 months. Lewerenz et al (1967) have reported that dogs exposed to this atmosphere for 8 months show changes in the lung which result in a decreased gas exchange surface, probably an adaptive response, and a thickening of the alveolar capillary barrier as a result of marked interstitial edema. Morphological changes have also been reported in liver (Schaffner, 1967) and kidney tissue (Mautner, 1966) primarily in the mitochondria, after exposure of rats to this atmosphere for several weeks. These tissues appear to return to normal after 90 days. The changes appeared similar to those found in animals exposed to 100% oxygen at ambient pressure although they appeared later and were less severe.

The objective of this investigation was to elucidate the time sequence of the pathogenesis of oxygen toxicity in various tissues, particularly defining the effects occurring after short exposure to 100% oxygen at ambient pressure that were similar to those occurring after long-term exposures at lower total pressures, and to correlate morphological changes with changes in cellular respiration, organ function, and blood oxygen levels.

Since morphological alterations in mitochondria can result in uncoupling of oxidative phosphorylation with a decrease in cellular adenosine triphosphate (ATP) production, both biochemical, physiological, and morphological parameters were measured. Mitochondrial

respiration and energy production in liver and kidney tissue, kidney function tests, blood gas levels, and histopathological examination of liver, kidney and lung tissue were determined on monkeys exposed to 100% oxygen at ambient pressure for 4, 7 and 12 days.

SECTION II

METHODS

Thirty-two monkeys, Macaca mulatta, ranging in weight from 2.7 to 5.1 kilograms were selected for exposure for the three time periods. All experiments were conducted in the Thomas Domes (see Thomas, 1965). These domes are dynamic flow experimental altitude chambers in which the environment is automatically controlled within narrow limits. An average pressure of 748 mm Hg was maintained and the oxygen content was between 99% and 100%. The exact dome operating conditions are described in Table I.

Table I

Dome Operating Conditions

	Average	Range
Pressure mm Hg	748	700-750
Flow Rate cu.ft./min.	20	18-35
Temperature °F.	77.6	74-81
Relative Humidity	59	42-69
CO_2	0.095%	0.00-0.44%

At a flow rate of 20 cfm there was a complete turnover of the atmosphere every 40 minutes. Daily entries were made into the dome through an air lock for feeding and cleaning procedures and for removing dead animals when necessary. The monkeys were housed in individual cages with an automatic watering system and

were fed once a day with Purina® monkey chow. Control animals were housed and treated in a similar manner outside the dome.

Blood for gas analyses was drawn under local anesthesia from the femoral artery of all animals at the end of their exposure period. P_{O_2} and P_{CO_2} levels were measured by an Instrumentation Laboratories Blood Gas Analyzer.

Twelve monkeys were selected for evaluation of kidney function and morphology. Each monkey served as his own control in this phase of the study. Tissue for electron microscopic examination was obtained by needle biopsy of the left kidney. To facilitate the biopsy procedure, the left kidney of each monkey was surgically translocated to a subcutaneous pocket (Yunis 1967, Kaplan 1967). Prior to exposure, baseline renal function tests and kidney biopsies were done on these monkeys. Glomerular filtration rate (GFR) was determined by endogenous creatinine clearance, effective renal plasma flow (RPF) by para amino hippurate (PAH) clearance, and tubular excretion by maximum PAH clearance (Tmax PAH) (Kaplan 1967). After baseline data were obtained, these monkeys were placed in the Thomas dome. Three groups of four monkeys each were exposed for 4, 7 and 12 days, respectively, and the function tests repeated. Biopsies were performed 24 hours after the function tests were completed.

Three other groups of monkeys were exposed for 4, 7, and 12 days to determine the effects of oxygen on liver and kidney cellular respiration, oxidative phosphorylation, and ATP levels. The monkeys were anesthetized with intravenous sodium pentobarbital, 30 mg/k. A medial abdominal incision was made to expose the liver and the

kidneys. For ATP analysis, tissues from both organs were frozen in situ as described by Hohorst et al (1959), and approximately one gram of tissue was quickly excised, dropped into liquid nitrogen and stored on dry ice in a freezer at -20°C. The ATP determinations were performed using the fluorimetric technique of Lowry (1964).

For liver and kidney mitochondrial isolation, approximately 4.5 g of liver and kidney tissue were excised, placed immediately in ice-cold sucrose, 0.25 M, and cut into 0.5 to 1.0 cm pieces. The mitochondria were prepared essentially by the method of Weinbach (1961) incorporating some modifications described by Nelson et al (1967). The chilled minced tissue was homogenized for about 30 seconds in ice-cold sucrose, 30% weight/volume (w/v), in a glass teflon tissue homogenizer. The homogenate was diluted 10% w/v with sucrose and centrifuged at 600 x g for 10 minutes and the supernatant decanted. The supernatant was then centrifuged at 8500 x g for 10 minutes and the supernatant decanted. The mitochondrial pellet was resuspended in sucrose and centrifuged again at 8500 x g for 10 minutes. This wash was repeated and the pellet was finally resuspended in sucrose at a concentration of 1 ml sucrose per 1 g original tissue wet weight. This mitochondrial suspension was kept at 0° C until used.

Mitochondrial oxygen uptake was determined manometrically by Warburg respirometry using a glucose hexokinase trap for phosphate. Both an α-ketoglutarate substrate, which undergoes pyridine nucleotide linked oxidation, and a succinate substrate, which does not, were used. Inorganic phosphate was measured by a modification of the Fiske Subbarow method Hawk (1947), and protein was measured by the Biuret

reaction. Oxygen consumption per unit nitrogen Q(O/N), phosphate uptake per unit nitrogen, P/N, and the ratio of phosphate uptake to oxygen consumption, P/O, were calculated from this data. Respiratory control ratios (RCR) were measured polarographically using the Gilson Oxygraph and a Yellow Springs Instrument Company Clark oxygen electrode. A constant-temperature, circulating water bath kept the temperature at 25°C ± 0.03°C. The mitochondrial suspension, 0.1 ml, was incubated in a medium, pH 7.4, containing per ml: 120 μmoles KCl, 20 μmoles glycylglycine, 8 μmoles $MgCl_2$, 5 μmoles orthophosphate, 10 μmoles substrate (alpha-ketoglutarate or succinate). Oxygen uptake of the mitochondrial suspension was measured for a short time in this condition, state 4, which is defined as respiration in the presence of excess substrate, orthophosphate and oxygen but without phosphate acceptor, ADP. Then 0.16 umole ADP/ml incubation medium was added and oxygen uptake measured in the presence of excess phosphate acceptor, state 3. This cycle was repeated two times. The respiratory control ratios, $\frac{Q(O/N)\ \text{State 3}}{Q(O/N)\ \text{State 4}}$ were obtained by averaging the second and third cycles of duplicate incubations; ADP/O ratios were also calculated from these data.

After the desired procedures were completed and while the monkeys from all exposure groups were still anesthetized, the lungs were perfused with glutaraldehyde buffered to pH 7.4 for morphological examination. A transverse incision was made in the trachea, and a cannula was inserted, tied, and attached to a bottle containing the glutaraldehyde. The bottle was fixed at such a height that at the start of the flow the hydrostatic pressure was standardized at 20 cm water. The

diaphragm was punctured, and the solution was allowed to fill the lungs to their maximum extensibility. The volume was measured and the trachea was tied off. The lungs were then dissected out of the chest cavity and placed in a glutaraldehyde bath. This procedure allows fixation to proceed quickly and the lungs do not collapse, thus providing improved sections for histopathological evaluation.

The animals were removed from the dome and necropsied.

SECTION III

RESULTS

Mortality: All of the monkeys showed signs of distress after 48 to 72 hours exposure. They became lethargic, refused food, and huddled to one side of their cage. Between 4 and 8 days exposure, the monkeys appeared quite ill and seemed in respiratory distress with labored breathing. Two of the five monkeys that survived 12 days exposure appeared to improve on the 9th day although the other surviving monkeys remained quite ill. Three animals had a frothy bloody exudate from the nose and mouth after 10 days exposure. Twelve of the thirty-two monkeys died during exposure. Twenty survived the planned exposure times and were sacrificed in the dome after the procedures were completed. The data on time of death or sacrifice and mortality are given in Table II.

Table II

Time of Death

	4 days	7 days	12 days
Number of monkeys sacrificed/time period	8	7	5
Mortality dead/number exposed	1/32	8/23	12/17

One monkey died on the 3d day, eight died between the 5th and 8th day and three died between the 10th and 12th day.

Blood Gases: The blood gas data are listed in Table III.

Table III

Blood Gases mm Hg

Control		4 days		7 days		12 days	
P_{O_2}	P_{CO_2}	P_{O_2}	P_{CO_2}	P_{O_2}	P_{CO_2}	P_{O_2}	P_{CO_2}
100	22.0	515	34.0	103	70.5	355	33.5
85	29.5	355	35.0	305	40.0	115	42.5
91	25.5	535	39.5	138	30.0	57	-
92	22.5	460	27.5	93	30.5	355	25.0
98	29.5	500	36.5	50	43.5	48	40.5
92	26.5	325	32.5	253	43.5		

Control P_{O_2} values range from 85 to 100 mm Hg and P_{CO_2} values from 22.0 to 29.5 mm Hg. The low control P_{CO_2} values probably are due to hyperventilation and increased blood lactic acid during the procedure for obtaining blood samples. All monkeys were hyperoxic after 4 days exposure with an increased P_{CO_2}; at 7 days the animals were hypoxic relative to their 4-day levels. The animal that showed a P_{CO_2} of 70.5 mm Hg died within an hour after the blood sample was taken. The P_{O_2} and P_{CO_2} values at 12 days were scattered, indicating an individual variability in response.

Kidney Function: The results of the baseline and post exposure kidney function studies are shown in Table IV. Unfortunately, only 5 of the 12 monkeys exposed survived the planned exposure period.

Table IV

	GFR ml/min/k		RPF ml/min/k		T_{max} PAH mg/min/k	
	Baseline	Post exposure	Baseline	Post exposure	Baseline	Post exposure
4 days	2.46	0.66	16.87	6.46	1.96	0.79
	2.76	1.09	11.98	13.38	1.52	1.86
7 days	3.43	2.56	27.61	34.00	2.67	2.48
12 days	2.48	2.58	14.30	16.52	3.29	3.50
	2.60	2.34	7.90	10.03	1.61	2.43

The first 4-day monkey listed had a significant decrease in the three parameters measured; the second four day monkey listed had a significant decrease in GFR. The other animals showed no significant change. Examination of the biopsy tissue by electron microscopy showed morphological changes, primarily in the mitochondria of the proximal tubules of the animals exposed for 4 and 7 days. These changes consisted of mitochondria with an absence of christae, degeneration of some mitochondria and an increased number of microbodies in the cells. The tissue from monkeys exposed for 12 days showed less severe changes, with an apparent return to more normal-appearing cells.

Cellular Respiration: A total of 16 monkeys were exposed for the various time periods for the studies on cellular respiration and energy production. Fifty per cent expired before completing the test intervals. Therefore we were able to evaluate only three monkeys at 4 days, three at 7 days, and two at 12 days. There were five control monkeys. The individual values for each animal and the average values for each exposure time group for ATP levels, P/O ratios, and P/N ratios in liver and kidney tissue are shown in Tables V, VI, and VII, respectively. The Q(O/N) values in

State 3 and State 4, the ADP/O and RC ratios are listed in Tables VIII, IX, X, and XI, respectively.

There appeared to be a trend toward decreased ATP levels with increased time of exposure. This is particularly evident in the kidney tissues. The changes in kidney ATP levels occurred earlier than in liver, and the changes were more uniform among the individual animals in each exposure group. The P/O and P/N ratios also showed a trend downward with increased time of exposure. The respiration rate, Q(O/N), in State 4, i.e. that occurring in the absence of ADP, appears to be elevated after prolonged exposure to oxygen. ADP/O values, in contrast to the P/O ratios obtained using Warburg Respirometry, are unchanged. There is a minimal depression of the RC ratios which is probably a reflection of the increased Q(O/N) in State 4.

Table V

ATP Concentration
μmoles/g

Liver

Control	4 days	7 days	12 days
2.6	3.6	3.6	1.7
1.5	2.1	2.7	2.9
3.8	2.4	2.3	
3.1			
3.6			
2.9	2.7	2.9	2.3

Kidney

0.7	1.9	1.6	1.1
1.7	0.9	1.5	0.9
2.7	1.1	1.1	
2.9			
2.4			
2.1	1.3	1.4	1.0

Table VI

P/O Ratio

	Liver		Kidney	
	∝ Ketoglutarate	Succinate	∝ Ketoglutarate	Succinate
Control	3.8	1.7	3.0	1.9
	3.1	1.7	2.9	1.9
	2.7	2.1	3.0	1.9
	2.3	2.3	3.4	2.7
		1.8	3.1	2.0
	3.0	1.9	3.1	2.1
4 Days	3.4	1.5	2.7	1.7
	2.6	1.5	2.2	1.7
	3.1		2.1	
	3.0	1.5	2.3	1.7
7 Days	2.0	1.6	2.0	1.5
	2.3	1.3	2.5	1.6
	3.1	1.4	2.4	1.5
	2.5	1.4	2.3	1.5
12 Days	2.4	1.5	2.5	1.6
	2.6	1.4	2.8	1.9
	2.5	1.4	2.7	1.8

Table VII

P/N Ratio

	Liver		Kidney	
	α Ketoglutarate	Succinate	α Ketoglutarate	Succinate
Control	8.5	10.0	20.0	17.0
	8.1	10.7	27.5	17.1
	9.7	11.5	28.6	19.7
	8.0	10.7	32.1	23.4
	11.8	6.9	22.7	15.4
	9.2	10.0	26.2	18.5
4 Days	4.5	7.9	17.9	14.0
	8.4	6.9	14.6	12.6
	5.3		9.7	
	6.1	7.4	14.1	13.3
7 Days	7.3	9.6	11.5	9.8
	6.0	7.8	20.8	15.5
	4.3	7.7	14.2	11.0
	5.9	8.4	15.5	12.2
12 Days	5.4	7.0	16.9	10.9
	8.4	9.3	24.1	16.0
	6.9	8.2	20.5	13.4

Table VIII

Q(O/N) State 3

	Liver		Kidney	
	α-Ketoglutarate	Succinate	α-Ketoglutarate	Succinate
Control	21.4	47.3	42.4	73.5
	20.5	46.0	50.3	73.9
	24.5	51.5	34.1	71.7
	23.2	48.0	56.8	84.1
	22.4	48.2	45.9	75.8
4 Days	19.8	48.5	44.1	84.9
	23.0	45.2	35.4	53.6
	21.0	51.2	28.8	60.4
	21.3	48.3	36.1	66.3
7 Days	16.1	48.6	18.5	42.7
	23.5	51.4	56.8	73.7
	19.7	45.5	41.1	63.7
	19.8	48.5	38.8	60.0
12 Days	22.3	43.1	54.2	75.7
	22.6	54.8	54.2	82.1
	22.5	48.9	54.2	78.9

Table IX

Q(O/N) State 4

	Liver		Kidney	
	∝ Ketoglutarate	Succinate	∝ Ketoglutarate	Succinate
Control	6.7	11.5	13.5	26.0
	5.9	10.3	14.2	23.9
	5.7	10.9	15.2	29.6
	6.3	10.6	16.2	27.8
	6.2	10.9	14.8	26.8
4 Days	6.4	11.2	10.5	20.7
	7.8	13.4	16.1	27.2
	7.7	13.6	14.4	25.2
	7.3	12.7	13.7	24.4
7 Days	5.2	11.8	9.9	17.4
	6.6	11.3	13.5	21.5
	7.7	12.6	12.9	20.9
	6.5	11.9	12.2	20.0
12 Days	8.4	14.7	22.4	37.3
	6.7	12.5	15.7	26.4
	7.6	13.6	19.0	31.9

Table X

ADP/O

	Liver		Kidney	
	α Ketoglutarate	Succinate	α Ketoglutarate	Succinate
Control	2.0	1.5	2.0	1.4
	2.2	1.5	2.1	1.5
	2.1	1.5	1.9	1.3
	2.1	1.5	2.1	1.5
	2.1	1.5	2.0	1.4
4 Days	2.0	1.4	2.0	1.4
	1.9	1.4	1.7	1.1
	1.8	1.4	1.7	1.3
	1.9	1.4	1.8	1.3
7 Days	1.9	1.5	1.7	1.3
	2.0	1.4	2.1	1.4
	1.9	1.3	2.1	1.4
	1.9	1.4	2.0	1.4
12 Days	1.7	1.5	1.7	1.2
	2.1	1.5	2.1	1.5
	1.9	1.5	1.9	1.4

Table XI

RC Ratio

	Liver		Kidney	
	∝-Ketoglutarate	Succinate	∝-Ketoglutarate	Succinate
Control	3.2	4.1	3.3	2.9
	3.5	4.4	3.6	3.1
	4.3	4.7	2.2	2.6
	3.7	4.5	3.5	3.2
	3.7	4.5	3.1	2.9
4 Days	3.1	4.4	4.2	4.1
	2.9	3.4	2.2	2.0
	2.7	3.8	2.0	2.4
	2.9	3.8	2.8	2.8
7 Days	3.1	4.4	1.9	2.5
	3.6	4.5	4.4	3.5
	2.5	3.6	3.2	3.0
	3.1	4.1	3.1	3.0
12 Days	2.7	2.9	2.4	2.1
	3.4	4.4	3.5	3.1
	3.0	3.7	3.0	2.6

Morphology: The most pronounced morphological changes were seen in the lung. However, 27 of the 32 monkeys also had pathological changes in the kidney. There was a mild to moderately severe nephritis with tubular swelling and some tubular necrosis. Some tubules were filled with an eosinophilic material and with hyaline casts. There was also a mild proteinuria. The severity of these changes did not appear to be correlated with time of exposure.

The lung pathology and time sequence of pulmonary morphological changes are described below. Most of the monkeys had a mild infestation of lung mites.

The one monkey that died on the 3d day of exposure had a marked alveolar edema with a small amount of alveolar exudate which was fibrinocellular in nature. There was a mild focal septal edema containing inflammatory cells and a mild peribronchiolar inflammation.

Eight animals were sacrificed after 4 days exposure. The larger vessels were mildly congested; there was a moderate focal peribronchiolar inflammation; and the lymphatics were distended. The septa had mild focal edema. Alveolar edema was found in varying intensity in all cases with an alveolar exudate mainly of fibrin but with some polymorphonuclear neutrophiles and red blood cells. There did not appear to be enough lung damage to cause any impairment of gas exchange across the alveolar capillary barrier.

Five animals died on the 5th and 6th day of exposure. These monkeys had a moderate amount of alveolar edema with a diffuse fibrinocellular alveolar exudate containing a few polymorphonuclear

cells and phagocytes. Several monkeys had focal hemorrhages in the alveoli. There was increased septal thickening with edema, inflammatory cells, and red blood cells. Several monkeys had hypertrophy and hyperplasia of the granulocytic pneumocytes. These monkeys showed no evidence of major impairment of gas exchange.

Nine monkeys were necropsied after seven days exposure. There was a marked septal thickening primarily due to fibrinoblasts with an increase in collagen and connective tissue. Fibrin and neutrophiles were scattered through the septa. Granulocytic pneumocytes were hypertrophic and hyperplastic. Alveolar edema and exudate containing fibrin, red blood cells and phagocytes were seen in all monkeys. Two monkeys had a moderately extensive emphysema. In six of the animals a marked degree of edema superimposed upon a large amount of organizing or consolidated fibrinocellular exudate was probably severe enough to cause impairment of gas diffusion.

Four animals died between the 8th and 12th day of exposure. There was a severe septal thickening with connective tissue, fibroblasts, edema and inflammatory cells. The granulocytic pneumocytes were both hypertrophic and hyperplastic. The severe alveolar exudate was fibrinocellular and in many areas it was difficult to differentiate between the septa and the exudate. Two of the monkeys had a moderately severe emphysema.

One animal that was sacrificed on the 12th day had only mild lung changes. There was a very minimal amount of alveolar edema and exudate. The septa were thickened by connective tissue but

the lungs appeared in fairly good condition. However, the other four monkeys exposed for 12 days had large areas consolidated with organized alveolar exudate composed of connective tissue, fibrin and cells, and heavily thickened septa separated by emphysematous areas. There was extreme hyperplasia and hypertrophy of granulocytic pneumocytes in areas not consolidated. These monkeys had grossly impaired gas diffusion.

In one animal, the exudate appeared to be resolving slightly. Areas of consolidation alternated with areas containing no exudate and moderately thickened septa in a ratio of approximately 3:1.

SECTION IV

DISCUSSION

The principal cause of death in these monkeys was apparently a severe hypoxia resulting from an impaired gas diffusion across the alveolar capillary barrier. Generally the lung pathology followed a pattern of mild congestion and inflammation at 4 days, an acute and severe alveolar edema with a massive amount of exudate at 7 days and, at 12 days, either a consolidation of exudate with emphysematous areas present or a resolution of the exudate with a thickened layer of connective tissue in the septa. The blood P_{O_2} levels reflected this pattern fairly well.

All P_{O_2} levels indicated an hyperoxia during the early stage with a relative hypoxia developing as the acute phase progressed and, finally, either an absolute hypoxia with the most severe lung changes or an increase in P_{O_2} in those animals able to resolve the exudate. This pattern generally followed that described by Robinson et al (1967); i.e. an acute "exudative" phase, characterized by a large degree of alveolar edema and exudate with septa thickened by fibrinocellular material, followed by a "proliferative" phase consisting of an increase in connective tissue in the septa with no edema or exudate. The authors considered this latter phase compatible with life in this atmosphere. They reported the time of onset of symptoms and the pathological changes as being most pronounced between 3 and 7 days of exposure.

In the experiment described above, the changes in the lung occurred more slowly and were most severe between 6 and 9 days exposure. Moreover only two of the monkeys exposed for 12 days gave any indication of being in the "proliferative" phase and one of these still had a severe alveolar exudate. We did not feel that either of these animals would survive this atmosphere indefinitely. The increasing layering of fibrin in the septa would eventually lead to a diffusion barrier of sufficient magnitude to cause hypoxia. Furthermore, in our study, the majority of the monkeys were unable to resolve the extreme amount of edema and exudate and died in the exudative phase. The difference in time of onset of symptoms and lung changes is probably due to individual animal variation.

The only other consistent pathological damage was observed in the kidney tissue. Both the light and electron microscopic examination of the kidney showed rather nonspecific but definite pathological changes occurring at 4 and 7 days exposure with an apparent reversed trend toward normal at 12 days.

Although the number of monkeys that had kidney function tests performed was extremely limited, the data tend to substantiate the morphological changes: decrease in GFR in two monkeys and in RPF and Tmax PAH in one monkey after four days, with no functional changes seen at 7 or 12 days. The same changes in kidney function at the same times of exposure were also seen in two monkeys exposed to 100% oxygen at 750 mm Hg in a previous experiment. The major damage to the kidneys occurs during the hyperoxic period with a

return to normal levels as the monkeys become progressively hypoxic. The P/O and P/N ratios in the kidney appeared to have a similar trend although the changes were not statistically significant. The kidney ATP levels, however, were still low after 12 days exposure. It may be that these would also tend to return to normal values if followed for a longer period of time.

The decrease in P/O ratios, P/N ratios and ATP levels were not severe enough in either kidney or liver tissue to cause death. The kidney tissue appeared to be more sensitive and to exhibit a more uniform response to the oxygen atmosphere than liver tissue. There is, however, no correlation between the level of P_{O_2} and degree of depression of cellular respiration. The animal with the greatest degree of uncoupling did not have the lowest P_{O_2}.

Although exposure to 100% oxygen for 4, 7, and 12 days did cause a certain amount of uncoupling of oxidative phosphorylation and decreased energy production in liver and kidney tissue as well as functional changes and morphological changes in the kidney, the major pathological changes occurred in the lung with the cause of death most probably due to severe hypoxia.

REFERENCES

Hawk, P.B., B.L. Aser, W.H. Summerson, Practical Physiological Chemistry, 13th Edition, The Blakiston Co., New York, N.Y., 1947.

Hohorst, H.J., F.H. Kreutz, and T. Bucher, "On the metabolite content and the metabolite concentration in the liver of the rat," Biochem, Z., Vol 332, p 18, 1959.

Kaplan, H.P., M.E. George, and J.L. Gillmore, "Kidney translocation for toxicologic evaluation," Proceedings of the Third Conference on Atmospheric Contamination in Confined Spaces, AMRL-TR-67-200, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, 1967.

Kistler, Gonzague S., P.R.B. Caldwell, and E.R. Weibel, Electron microscopic and morphometric study of rats exposed to 98.5 percent oxygen at atmospheric pressure, AMRL-TR-65-66, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, 1965.

Klion, F.M., F. Schaffner, and H.P. Kaplan, "Hepatic effects of breathing pure oxygen for eight months upon rats, dogs, and monkeys," Aerospace Medicine, Vol 38 (3), p 273, 1967.

Lewerenz, M., G. Schwinger, and E.R. Weibel and H.P. Kaplan, "Electron microscopic and morphometric evaluation of lungs from animals exposed continuously for eight months to 5 psia, 100% oxygen," Proceedings of the 3rd Annual Conference on Atmospheric Contamination in Confined

Spaces, AMRL-TR-67-200, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, 1967.

Lowry, O.H., J.V. Passonneau, F.X. Hasselberger, and D.W. Schulz, "Effects of ischemia on known substrates and cofactors of the glycolytic pathway in brain," Journal Biological Chemistry, Vol 239 (1), p 18, 1964.

Mautner, Willie, "Electron microscopic investigation of oxygen effects on kidney tissue," Proceedings of the Second Annual Conference on Atmospheric Contamination in Confined Spaces, AMRL-TR-66-120, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, 1966.

Nelson, B.D., B. Highman, and P.D. Altland, "Oxidative phosphorylation during altitude acclimation in rats," American Journal Physiology, Vol 213, p 1414, 1967.

Riesen, W.H., J.Q. Kissane, R.W. Bieber, M.L. Kardatzke, H.P. Kaplan, and P. Felig, "Research on Oxygen Toxicity at the Cellular Level," AMRL-TR-66-206, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, 1966.

Robinson, F.R., D.T. Harper, A.A. Thomas, and H.P. Kaplan, "Proliferative pulmonary lesions in monkeys exposed to high concentrations of oxygen," Aerospace Medicine, Vol 38, p 481, 1967.

Schaffner, Fenton and Philip Felig, "Changes in hepatic structure in rats produced by breathing pure oxygen," The Journal of Cell Biology,

Vol 25 (3), p 505, 1965.

Thomas, Anthony A., "Chamber Equipment Design Considerations for Altitude Exposures," Proceedings of the Conference on Atmospheric Contamination in Confined Spaces, AMRL-TR-65-230, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, 1965.

Weinbach, E.C., "A procedure for isolating stable mitochondria from rat liver and kidney," Analytical Biochemistry, Vol 2, p 335, 1961.

Weir, F.W., D.W. Bath, P. Yevich, F.W. Aberst, A study of the effects on continuous inhalation of high concentrations of oxygen at ambient pressure and temperature, ASD Technical Report 61-664, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, 1961.

Yunis, S.L., V.A. DeScala, C. Oh, F. Lomita, and J.H. Jacobson II, "The subcutaneous kidney: Simple technique for serial biopsy and renal function studies," Investigative Urology, Vol 5 (3), p 313, 1967.